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Enhancement of Salvage of Reperfused Myocardium by Early Beta-Adrenergic Blockade (Timolol)

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Although reperfusion of severely ischemic myocardium with thrombolytic agents or surgery has shown reduction in infarct size, the time after coronary occlusion during which reperfusion can salvage ischemic myocardium is limited. To determine whether beta-adrenergic blockade could enhance the salvage of ischemic myocardium by reperfusion, the left anterior descending coronary artery was occluded in 18 anesthetized dogs. An in vivo area at risk was determined by injecting technetium-99m–labeled albumin microspheres into the left atrium 5 minutes after occlusion and carrying out radioautography to define the poorly perfused tissue. Fifteen minutes after coronary occlusion, the dogs were randomized either to a control (saline-treated) group (n = 8) or to a timolol-treated group (n = 10). Timolol was administered until a decrease of 20% in heart rate or blood pressure occurred (mean total dose = 0.85 ± 0.22 mg/kg ± standard error of the mean). Coronary occlusion was maintained for 3 hours and was followed by 3 hours of reperfusion in both groups. At the end of 6 hours, infarct size was defined by triphenyltetrazolium chloride staining and masses of infarct and risk were calculated.

Percent left ventricular mass at risk was similar for both groups (control = 20.9 ± 2.4%, timolol-treated = 23.7 ± 2.1%, p = not significant). Mass of necrosis/mass at risk was significantly smaller in the timolol-treated reperfusion group (27.3 ± 2.7%) versus saline reperfusion alone (46.5 ± 5.6%) (p < 0.005). Thus, beta-adrenergic blockade administered early after coronary occlusion results in substantial enhancement of the salvage achieved by reperfusion alone.

After sudden obstruction of a coronary artery, the myocardium perfused by the occluded vessel immediately ceases to contract. A wavefront of necrosis commences in the subendocardium and for several hours proceeds outward to reach the subepicardium; in the anesthetized dog, reperfusion carried out within 15 minutes of occlusion generally salvages all of the ischemic myocardium (1,2). As the time after occlusion becomes prolonged, the quantity of tissue salvaged by reperfusion rapidly diminishes, so that by 3 hours only the subepicardial myocardium can be salvaged by reperfusion (2,3) and by 6 hours essentially all of the tissue in the area of distribution of the occluded vessel has become necrotic (4,5). The demonstration that a coronary thrombus can be lysed by means of intracoronary or intravenous streptokinase has led to intense interest in the use of reperfusion to salvage ischemic myocardium (6–8). As might be expected from experiments in the dog, it has also been observed clinically that the time interval after coronary occlusion during which reperfusion will salvage significant myocardium is relatively brief. Indeed, most clinical investigators (7–9) believe that little if any myocardium can be salvaged if reperfusion is delayed by more than 4 or 5 hours after the onset of the clinical events associated with infarction. Because it is often difficult for logistic reasons to carry out reperfusion within this short time interval, it would be extremely desirable to enhance the quantity of myocardium salvaged by reperfusion carried out several hours after coronary occlusion.

Beta-adrenergic blocking agents have been shown to diminish the severity of myocardial ischemic injury after coronary occlusion (10–24), although it is unknown whether these agents alone can actually reduce the mass of the myocardial infarct after a permanent coronary occlusion (25–27). In view of the salutary effects of beta-adrenergic blockade

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on ischemic myocardium, we wished to determine whether this intervention might delay cell death sufficiently so as to enhance the extent of myocardial salvage by reperfusion carried out several hours after coronary occlusion.

Methods

Experimental preparation. Mongrel dogs of both sexes weighing between 9 and 27 kg were sedated with acepromazine maleate (1.0 mg/kg body weight subcutaneously), anesthetized with pentobarbital (30 mg/kg intravenously) and intubated and placed on a Harvard respirator (Ealing Co.). A thoracotomy was performed in the fifth left intercostal space, the lungs were retracted and the heart was supported in a pericardial cradle. Polyvinyl catheters were placed in the femoral vein for fluid and drug administration, in the femoral artery for blood pressure recording and withdrawal of microsphere reference samples and in the left atrium for injection of radioactive microspheres. A micromanometer-tipped pressure catheter (Millar Instruments) was placed into the left ventricle through the apex.

All animals were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources, National Research Council (DHEW publication No. [NIH] 78-23, revised 1978).

Experimental protocol. After administration of lidocaine (1.5 mg/kg intravenously), the left anterior descending coronary artery was occluded with a Schwartz vascular clamp. A second similar dose of lidocaine was administered 5 minutes after coronary artery occlusion. Five minutes after occlusion, 600,000 technetium-99m–labeled human albumin microspheres (dose 0.54 mCi/kg) were injected slowly into the left atrium for determination of area at risk by autoradiography as previously described by DeBoer et al. (4). Radioactive plastic microspheres (cerium-141, scandium-46, stannum-113) were injected for determination of regional myocardial blood flow 10 minutes after occlusion, as previously described (28). Fifteen minutes after coronary occlusion the dogs were randomized either to a control group ($n = 8$) receiving saline infusion or to a group ($n = 10$) treated with timolol maleate (Merck Sharp and Dohme, Research Lab) until a decrease of approximately 20% in heart rate or systolic arterial pressure was detected (mean total dose 0.85 ± 0.22 mg/kg ± standard error of the mean). Coronary occlusion was maintained for 3 hours and was followed by 3 hours of reperfusion by releasing the clamp in both groups. Heart rate and arterial and left ventricular pressure were continuously recorded before occlusion, throughout occlusion and during reperfusion periods.

Echocardiographic analysis. Two-dimensional echocardiograms were obtained from the open chest dogs with an ATL Mark III model echocardiograph with an 850A real time scan controller. Images were recorded on Scotch (3M) videocassettes with a Panasonic NV-8200 recorder. A saline-filled glove was placed between the epicardium and the transducer to place the epicardial surface within the focal zone. Short-axis echocardiographic images were traced directly from the video display (ATL 315A) from a stop-frame analysis of three consecutive cardiac cycles. These tracings were taken at end-diastole and end-systole with the onset of the Q wave in lead II to define end-diastole and the peak of the T wave to define end-systole. The images were traced by an investigator who was unaware of the treatment the dogs had received. Short-axis images for calculation were taken from the center of the infarcted zone, which was clearly visible at the level of the papillary muscles. For studies of short-axis change, end-diastolic area was measured by planimetry from the maximal short-axis cross section at the end of diastole; end-systolic area was determined at the same location.

Percent change of area (% ΔA) was calculated as follows:

$$\% \Delta A = (EDA - ESA)/EDA,$$

where EDA = end-diastolic area and ESA = end-systolic area. Two-dimensional echocardiograms were obtained 2 hours after occlusion and toward the end of the experiment.

Postmortem studies. The animals were killed with an overdose of potassium chloride to arrest the heart in diastole and the heart was excised. The left ventricle was dissected free, and its surfaces sprayed with liquid Freon to allow easy sectioning; the left ventricle was then sectioned parallel to the atrioventricular groove in 5 mm transverse slices.

The size of the myocardial infarct was determined by incubation in 1% triphenyltetrazolium chloride for 10 minutes at 37°C. Infarcted myocardium appears pale gray, whereas normal myocardium stains deep brick red (29,30). Autoradiography of each myocardial section was performed as previously described (4). With this technique, normally perfused myocardium appears as an area of increased radiographic density, whereas ischemic areas appear as clearly demarcated zones of decreased radiographic density ("cold spot images"). Tracings of each heart section were superimposed on the autoradiograph and the border of the ischemic area at risk was drawn at the edge of the zone of decreased radiographic density. The areas of necrosis and risk were determined by planimetry and corrected to the weight of the heart slices and expressed as mass of necrosis and mass at risk; infarct size was expressed as mass of necrosis as a percent of the mass at risk.

Statistical analysis. Unpaired *t* tests were used to calculate the significance of differences of masses of necrosis and risk, hemodynamic measurements and echocardiographic data between the groups. Values were expressed as mean ± standard error of the mean.

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Results

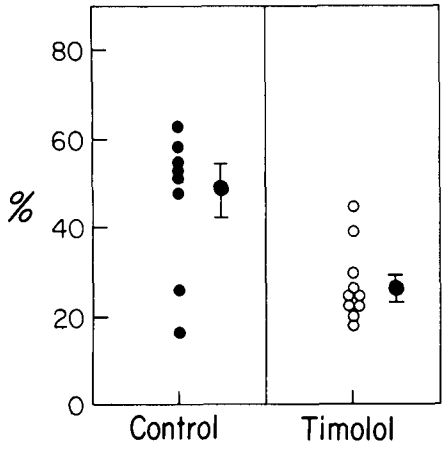
Twenty-seven dogs were entered into the protocol. Seven dogs developed ventricular fibrillation shortly after occlusion before randomization and died. One dog in the control group developed hypotension and died 30 minutes after occlusion. Another dog in the timolol-treated group required multiple defibrillations and was excluded from the study. Of the remaining 18 dogs that survived for 6 hours, 8 were in the control group (reperfusion alone) and 10 were in the treated group (timolol plus reperfusion).

Assessment of myocardial salvage (Fig. 1). Percent left ventricular mass at risk as assessed by autoradiography was similar in both groups (control 20.9 ± 2.4% and timolol-treated 23.7 ± 2.1%, probability [p] = not significant [NS]). Mass of necrosis expressed as a percent of mass at risk was 46.5 ± 5.6% in the control group and significantly smaller, 27.3 ± 2.7%, in the timolol-treated group ($p < 0.005$). Thus, timolol treatment followed by myocardial reperfusion results in substantial enhancement of myocardial salvage achieved by reperfusion alone.

Regional myocardial blood flow. Five minutes after occlusion, subendocardial and subepicardial flows were similar in both groups; in the ischemic zone these values were 0.18 ± 0.04 and 0.33 ± 0.11 ml/min per g, respectively, in the control group and 0.16 ± 0.05 and 0.20 ± 0.11 in the timolol-treated group (p = NS between corresponding regions in the two groups). In the nonischemic zone, subendocardial and subepicardial flows were 1.18 ± 0.11 and 0.88 ± 0.12, respectively, in the control group and 1.52 ± 0.46 and 1.18 ± 0.22 in the timolol-treated group (p = NS).

Hemodynamic measurements (Table 1, Fig. 2). Mean heart rate in the preocclusion period was similar in both groups (140 ± 8 beats/min) in the control group and 141 ± 7 in the timolol-treated group (before treatment) (p = NS). The percent decrease in heart rate in the timolol-treated group was 15.6 ± 3.2% at 2 hours after occlusion; in the control group the decrease was significantly less (5.4 ± 3.4%) ($p < 0.05$). Mean systolic pressure before occlusion was 114.8 ± 8.4 mm Hg in the control group, and 125.3 ± 8.3 in the timolol-treated group (before treatment) (p = NS). The percent decrease in systolic pressure was significantly greater in the timolol-treated group (22.6 ± 3.3%) than in the control group (9.2 ± 3.1%) ($p < 0.01$). The rate-pressure product (heart rate × peak systolic pressure) decreased from preocclusion values by a mean of 35.1 ± 2.9% in the timolol-treated group at 2 hours after occlusion; in the control group the decrease was smaller, 14.5 ± 2.6% ($p < 0.001$). Although left ventricular end-diastolic pressure tended to be higher in the timolol-treated group, this difference did not approach statistical significance.

Figure 1. Percent mass of necrosis over mass at risk for control dogs **(closed circles)** and for timolol-treated dogs **(open circles). Circles with bars** are mean values ± standard error of the mean. Differences between the two groups were significant ($p < 0.005$).



Echocardiographic measurements. Echocardiographic measurements were obtained at 2 hours after occlusion and 5 to 6 hours after occlusion (2 to 3 hours of reperfusion) in seven control and nine timolol-treated dogs. Short-axis area change calculated from images at the center of the infarct at 2 hours after occlusion was 27.5 ± 4.7% in the control group and 34.6 ± 2.8% in the timolol-treated group (p = NS). Postreperfusion (2 to 3 hours) short-axis area change was 30.4 ± 4.3% for the control group and 36.9 ± 2.8% for the timolol-treated group (p = NS). Thus, the beta-adrenergic blocking actions of timolol did not cause significant impairment of regional function.

Discussion

Recently, it has become apparent that early restoration of blood flow after coronary occlusion may result in limitation of myocardial necrosis in patients with evolving myocardial infarction (6–8). The rate of progression of necrosis may be influenced by the residual collateral circulation to the ischemic zone as well as hemodynamic and metabolic factors affecting myocardial oxygen demand. Because there is an inevitable delay between the clinical events usually signifying myocardial infarction and the time at which myocardial reperfusion can be started, the possibility of combining a pharmacologic intervention that reduces myocardial oxygen demands with myocardial reperfusion was considered.

Beneficial effects of timolol. The present study demonstrates that beta-adrenergic blockade achieved by early timolol administration followed by myocardial reperfusion results in substantial enhancement of myocardial salvage achieved by reperfusion alone; the percent of myocardium salvaged by reperfusion 3 hours after coronary occlusion increased from an average of 53% in the control dogs to 73% in the dogs treated with timolol 15 minutes after coronary occlusion and reperfused 3 hours later.

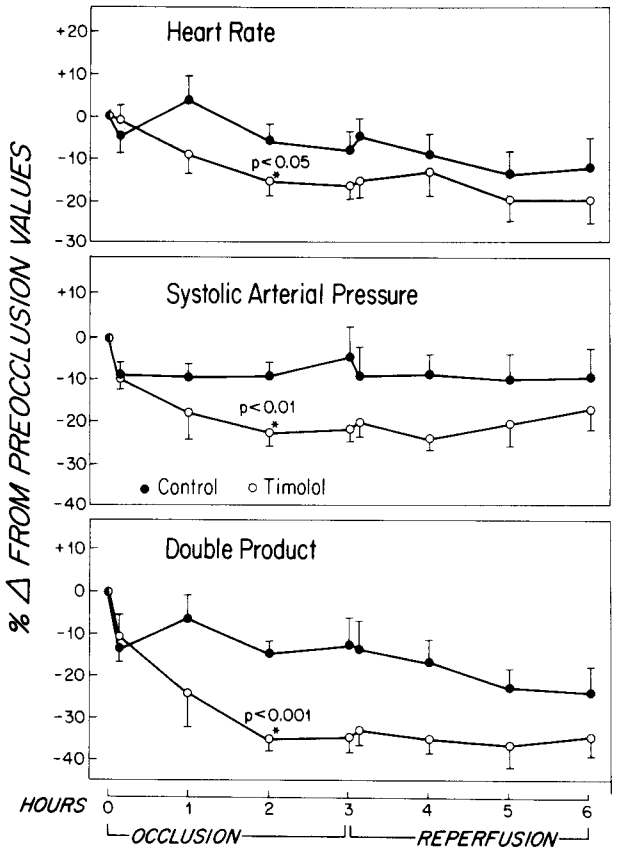


Figure 2. Percent change from preocclusion (100%). Values from top to bottom are for heart rate, systolic arterial pressure and double product (heart rate × systolic arterial pressure). **Closed circles with bars** are mean values ± standard error of the mean for control dogs; **open circles with bars** are mean values ± standard error of the mean for timolol-treated dogs.

Timolol maleate is a beta-adrenoreceptor blocking drug that lacks both intrinsic sympathomimetic activity and membrane stabilizing properties (31). The drug's half-life in plasma is approximately 3 to 4 hours. The level of beta-adrenergic blocking activity varies widely, and no simple relation exists between the dose of timolol, the plasma level and the therapeutic activity. Therefore, in our study timolol was administered not as a fixed dose, but according to a predefined hemodynamic response. This enabled us to determine the optimal dose for decreasing oxygen demands without causing deleterious hemodynamic effects. Experimental and clinical studies (31,32) have demonstrated that timolol reduces heart rate, left ventricular contractility and indexes of cardiac work.

Experimental studies in cats (33) have shown that timolol can protect ischemic myocardium as reflected in a reduced release of myocardial creatine kinase activity and reduction in ST segment elevation after coronary occlusion. Under controlled hemodynamic conditions, timolol appears to improve perfusion of the normal myocardium, whereas blood flow to ischemic tissue remains unchanged (34). Timolol has been reported to exert an antianginal effect in patients (35) and reduce mortality and reinfarction rates in those surviving acute myocardial infarction (36). However, not all beta-adrenergic blocking agents have been shown to have a beneficial effect on infarct size. In some studies (37,38), propranolol did not reduce infarct size.

Effects of timolol on regional function. An important question was how beta-adrenergic blockade affects regional function of the jeopardized zone. Whereas the negative inotropic effects exerted by beta-adrenergic blockade would be expected to impair function, the increase in tissue salvage induced by the beta-blocker might be expected to enhance ventricular function despite the anticipated postischemic depression of left ventricular function, that is, "stunning" of the myocardium (39). These two opposing influences appeared to balance each other and regional function did not differ in the control and timolol-treated groups when measurements were made at 2 hours after occlusion and at the end of the reperfusion period. We observed previously

Table 1. Hemodynamic Data*

	Preocclusion	2 Hours Postocclusion	6 Hours Postocclusion (3 hours postreperfusion)
Heart rate (beats/min)			
Control	139.8 ± 7.5	132.0 ± 8.0	127.0 ± 7.8
Timolol	141.0 ± 7.0	118.3 ± 6.4	111.6 ± 8.1
Systolic pressure (mm Hg)			
Control	114.8 ± 8.4	102.9 ± 4.6	101.4 ± 4.4
Timolol	125.3 ± 8.3	95.3 ± 3.9	101.0 ± 2.3
Left ventricular end-diastolic pressure (mm Hg)			
Control	6.0 ± 0.7	7.3 ± 1.2	8.8 ± 2.2
Timolol	4.6 ± 1.0	11.4 ± 4.4	9.0 ± 4.1

*The differences by grouped *t* test were not statistically significant in each time period between groups. Data are presented as mean values ± standard error of the mean. When data were expressed as percent change from preocclusion values, there were significant differences between the two groups (see text and Fig. 2).

(40) that although seriously ischemic tissue exhibits delayed postischemic recovery of function and high energy phosphate metabolism, significant recovery does, in fact, ultimately occur. In dogs subjected to 2 hours of coronary occlusion and 2 weeks of reperfusion, active left ventricular wall thickening occurs after 3 days of reperfusion (40). Other studies have shown that brief 15 minute temporary periods of ischemia not associated with necrosis result in substantial depression of adenosine triphosphate for at least 72 hours after reperfusion (41); however, adenosine triphosphate is fully recovered by 7 days (42). Therefore, it is likely that had functional measurements been made 1 to 2 weeks after reperfusion, the salvaged myocardium would have been superior in the dogs treated with timolol and reperfusion with their smaller infarcts.

Clinical implications. Beta-adrenergic blockade with timolol begun shortly after coronary occlusion followed by reperfusion resulted in substantial enhancement of myocardial salvage compared with reperfusion alone. Beta-adrenergic blockade might augment the quantity of tissue salvaged by reperfusion carried out early (within 4 hours of the coronary occlusion) and/or prolong the time interval after occlusion during which reperfusion may salvage severely ischemic myocardium that would otherwise be expected to undergo necrosis.

No animal model can exactly mimic the clinical situation; however, these observations have interesting clinical implications. The immediate administration of cardioprotective drugs, such as beta-adrenergic blocking agents, calcium channel antagonists or a variety of other agents that can delay cell death (10), might be desirable in patients with evolving myocardial infarction who are candidates for coronary reperfusion. Although in our study timolol was administered earlier after coronary occlusion than occurs in a clinical setting, the data have clinical relevance because there is a large group of patients with evolving myocardial infarction who are already receiving long-term beta-blocking therapy for angina and hypertension. In addition, there are now a group of patients at high risk of acute myocardial infarction who might receive beta-adrenergic blocking drugs prophylactically, especially if they have already had a previous infarction. Our results suggest that it is worth pursuing experiments in which beta-adrenergic blockade is administered a few hours after occlusion but before reperfusion to mimic current clinical coronary reperfusion studies.

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